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**BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES**

Application Number: 10/656,531
Filing Date: September 05, 2003
Appellant(s): BALTIMORE ET AL.

Dahna S. Pasternak
For Appellant

**EXAMINER'S ANSWER**

This is in response to the appeal brief filed 3/31/2009 appealing from the Office action mailed 1/9/2009.

**(1) Real Party in Interest**

A statement identifying by name the real party in interest is contained in the brief.

**(2) Related Appeals and Interferences**

The Examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

**(3) Status of Claims**

The statement of the status of claims contained in the brief is correct. Pursuant to 37 CFR 41.33(b)(1), Appellant has requested cancellation of claim 40. Since the cancellation of claim 40 does not affect the scope of any other pending claim in the proceeding, Appellant's amendment has been entered. Thus, the appealed claims are now claims 21, 28, 99-104, 107-108. Claims 43, 109-113, 120-135, 137-143 remain withdrawn from consideration.

**(4) Status of Amendments After Final**

The appellant's statement of the status of amendments after final rejection contained in the brief is correct. As indicated above, Appellant has requested the cancellation of claim 40 in the brief filed 3/31/2009. This amendment has been entered.

**(5) Summary of Claimed Subject Matter**

The summary of claimed subject matter contained in the brief is correct.

**(6) Grounds of Rejection to be Reviewed on Appeal**

The appellant's statement of the grounds of rejection to be reviewed on appeal is correct.

**(7) Claims Appendix**

The copy of the appealed claims contained in the Appendix to the brief is correct.

**(8) Evidence Relied Upon**

Bibikova et al., "Stimulation of Homologous Recombination through Targeted Cleavage by Chimeric Nucleases" Molecular and Cellular Biology, vol 21, no. 1 (January 2001), pp. 289-297

Takeuchi et al., "Flp recombinase transgenic mice of C57BL/6 strain for conditional gene targeting" Biochemical and Biophysical Research Communications, vol 293, (2002), pp. 953-957

**(9) Grounds of Rejection**

The following ground(s) of rejection are applicable to the appealed claims:

***Claim Rejections - 35 USC § 103***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 21, 28, 99-104, 107-108 are rejected under 35 U.S.C. 103(a) as being unpatentable over Choulika et al. (U.S. Publication No. 20020107214, U.S. Application No. 10/917295 filed on 7/27/2001) in view of Bibikova et al. (Molecular and Cellular Biology 21(1):289-297, 2001) and further in view of Takeuchi et al. (Biochemical and Biophysical Research Communications 293:953-957, 2002).

Choulika et al. teach (1) chimeric nucleases where the DNA binding domains are zinc finger binding domains, or meganuclease recognition sites, and the DNA cleavage domains are domains from restriction endonucleases, and (2) a chimeric nuclease comprising the DNA binding domain of a I-Sce I nuclease and the cleavage domain of a FokI nuclease ([paragraph [0042]). Choulika et al. teach that their invention relates to a method of repairing a specific sequence of interest in chromosomal DNA of a cell and teach a targeting DNA that comprises (1) DNA homologous to the region surrounding the site to be targeted in chromosomal DNA, and (2) DNA which repairs the specific sequence of interest upon recombination between the targeting DNA and the chromosomal DNA (paragraph [0026]). It is noted that the term "targeting DNA" of Choulika et al. is equivalent to the term "repair substrate" recited in the claims as both comprise (1) a DNA homologous to the region surrounding the site to be targeted in chromosomal DNA (i.e., nucleic acid sequence that is substantially identical to a region flanking a target sequence in chromosomal DNA), and (2) a DNA which repairs the specific sequence of interest upon recombination (i.e., nucleic acid sequence that replaces the target sequence upon recombination). Choulika et al. also teach a vector comprising a nucleic acid encoding the chimeric nuclease and the targeting DNA (paragraph [0049]; paragraph [0044]). Choulika et al. teach that the vector is a viral vector (paragraph [0045]), the vector has an inducible promoter (paragraph [0046]), and an isolated mammalian cell comprising said vector (paragraph [0052]). Choulika et al. do not disclose the use of a nuclear localization signal or a vector comprising a DNA encoding a second chimeric nuclease.

Bibikova et al. discloses that when cleavage of a chromosomal target is desired, it is very unlikely that exact inverted repeats of a 9-bp sequence will be located in favorable positions, thus there is a need to devise nucleases with two different sets of zinc fingers designed to bind two different 9-mers (page 294, left column, Cleavage of paired nonidentical recognition sites). Bibikova et al. teach the use of two chimeric nucleases with different binding specificities (Zinc finger DNA binding domains) that dimerize and together collaborate to stimulate recombination when their individual sites were appropriately placed

(Abstract; Materials and Methods, Enzymes). The chimeric nucleases are hybrids between the cleavage domain of FokI and a DNA binding domain made up of three $Cys_2$-$His_2$ zinc fingers (page 289, last paragraph). Bibikova et al. further disclose that because the recognition of DNA by zinc fingers is modular (each finger contacts primarily three consecutive base pairs in the target), they have been modified to create combinations with novel specificities (page 289, last paragraph). According to Bibikova et al., randomization of the codons for the recognition residues allows selection of new fingers that have high affinity for arbitrarily chosen DNA sequences (page 289, last sentence). Bibikova et al. tech that engineered zinc fingers have been shown to act on their designed targets in living cells and that nucleases based on zinc fingers should be targetable to specific but arbitrary recognitions sites (page 290, first 6 lines). Bibikova et al. teach that for specific cleavage to occur, dimerization of the cleavage domains from both chimeric nucleases is necessary, thus the two recognition sites for the zinc fingers must occur in close proximity (Abstract; page 295, left column, first full paragraph). Bibikova et al. teach that the chimeric endonucleases based on zinc fingers are capable of finding their recognition sites in oocytes, directing specific cleavage, and stimulating local homologous recombination (page 290, right column, first full paragraph). Bibikova et al. also teach the direct injection of these chimeric nucleases and DNA substrates for recombination directly into the nucleus of oocytes (Materials and Methods, Oocyte injections). Bibikova et al. teach that because the recognition specificity of zinc fingers can be altered experimentally, their approach holds great promise for inducing targeted recombination in a variety of organisms (Abstract, last sentence). Bibikova et al. do not teach a single vector comprising DNA encoding the two chimeric nucleases and the DNA substrate.

Takeuchi et al. teach a vector comprising DNA encoding Flp recombinase linked to a nuclear localization signal to increase the efficiency of the recombination process (page 954, right column, Results). Takeuchi et al. do not teach a vector comprising DNA encoding one or two chimeric nucleases and a DNA substrate.

Claims 21, 99-101 are directed in part to a viral vector comprising a DNA encoding a chimeric nuclease, wherein said chimeric nuclease comprises a zinc finger DNA binding domain, a nuclear localization signal, and a FokI cleavage domain, wherein the DNA encoding the chimeric nuclease is operably linked to an inducible promoter, and wherein said viral vector further comprises a DNA repair substrate, wherein said substrate comprises a nucleic acid sequence which is substantially identical to a region flanking a target sequence in chromosomal DNA, and wherein said substrate also comprises a nucleic acid sequence which replaces the target sequence upon recombination between the repair substrate and the target nucleic acid. Claim 102 is directed in part to the vector of claim 21 as described above with the added limitation that the vector further comprises a nucleic acid encoding a second chimeric nuclease, wherein the second chimeric nuclease forms a heterodimer with said chimeric nuclease. Claims 28, 103-104, 107-108 are directed to an isolated mammalian cell comprising a DNA encoding a chimeric nuclease, wherein said chimeric nuclease comprises a zinc finger DNA binding domain and a FokI cleavage domain, wherein the DNA encoding the chimeric nuclease is operably linked to an inducible promoter, and wherein said mammalian cell further comprises a DNA repair substrate, wherein said substrate comprises (1) a nucleic acid sequence which is substantially identical to a region flanking a target sequence in endogenous chromosomal DNA, and (2) a nucleic acid sequence which replaces the target sequence upon recombination between the repair substrate and the target nucleic acid.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the viral vector of Choulika et al. such that it would (1) encode one or two chimeric nucleases (as taught by Bibikova et al.) wherein the chimeric nucleases comprise a zinc finger DNA binding domain, a FokI cleavage domain, and a nuclear localization signal (taught by Takeuchi), wherein the nucleic acid encoding the chimeric nucleases is operably linked to an inducible promoter. Also, it would have been obvious to one of ordinary skill in the art at the time the invention was made to transform an isolated mammalian cell with the viral vector described above.

A person of ordinary skill in the art is motivated to (1) add a DNA encoding a nuclear localization signal to the vector of Choulika et al. in view of the teachings of Takeuchi et al., who disclose that adding a nuclear localization signal enhances recombination, (2) use chimeric nucleases which comprise zinc finger DNA binding domains and the FokI cleavage domain in view of the teachings of Choulika et al., who disclose chimeric nucleases comprising zinc finger DNA binding domains as well as chimeric nucleases having the FokI cleavage domain, and also in view of the teachings of Bibikova et al., who teach chimeric nucleases comprising both zinc finger DNA binding domains and the FokI cleavage domain, and (3) use two chimeric nucleases both having zinc finger DNA binding domains and the FokI cleavage domain in view of the teachings of Bibikova et al. who disclose that using two chimeric nucleases having two sets of three zinc binding fingers, allows for the possibility of directing cleavage to many more targets, and (4) use a single vector to deliver the nucleic acids encoding the chimeric nucleases and the repair substrate to a host cell for the benefit of delivering all the required components in a single vehicle thus reducing the number of transformation/transduction events to one. Also, a person of ordinary skill in the art is motivated to introduce the vector of Choulika et al., Bibikova et al. and Takeuchi et al. in an isolated mammalian cell for the benefit of targeted recombination, in view of the teachings of Choulika et al. (paragraph [0004]) and Bibikova et al. (page 289, left column, second paragraph), who teach that making an intentional double strand break in target DNA increases homologous recombination events.

One of ordinary skill in the art has a reasonable expectation of success at making the vectors and transforming isolated mammalian cells with said vectors since (1) Choulika et al. and Takeuchi et al. teach the construction of vectors comprising DNA encoding chimeric nucleases and nuclear localization signals, respectively, (2) Choulika et al. already teach a vector that comprises both the nucleic acid encoding the chimeric nuclease and the repair substrate, (3) Bibikova et al. teach vectors encoding each of the chimeric nucleases comprising Zinc finger DNA binding domains, and (4) the molecular biology

techniques required to make vectors and transform isolated mammalian cells with said vectors are well known and widely used in the art. Therefore, the invention as a whole would have been prima facie obvious to a person of ordinary skill in the art at the time the invention was made.

**(10) Response to Argument**

**I. Vectors carrying both a coding sequence (chimeric nuclease) and a repair substrate sequence are non-obvious over the cited references.**

On pages 5-7 of the brief, Appellant argues that the references teach that the coding sequences and repair (non-coding) sequences should be separated onto separate vectors, and that the separation of these sequences ensure that there is no interference between the two sequences. Appellant argues that the Examiner has not provided any evidence that using a single vector is simpler or beneficial, and instead has admitted on the record that the cited references do not teach a single vector. According to Appellant, the skilled artisans working in the field of homologous recombination specifically chose to separate the coding sequences and the non-coding sequences onto different vectors because (1) they believed it would be beneficial and simpler to keep them separate, and (2) they did not want expression of the nuclease to interfere with access to the repair substrate or vice versa. According to appellant, the evidence establishes that the skilled artisan would have thought including both sequences on the same vector would complicate matters and be detrimental to their individual functions. It is Appellant's contention that there is absolutely no motivation or expectation of success from the references that placing both sequences in one vector would be simpler or beneficial. Appellant submits that if this were to be the case, the artisans in the field at the time of filing would have used such a single vector. Appellant contends that these artisans did not use a single vector because there was no expectation that such vector would be functional. Appellant, citing *KSR International Co. v. Teleflex Inc.*, Fed. Reg. Vol. 72, No. 195, October 10, 2007, submits that the claimed invention is not obvious because the evidence establishes that

combining the chimeric nuclease coding sequence with the repair substrate sequence onto a single vector is unpredictable in terms of expression of the coding sequence and/or availability of the repair substrate.

Appellant's arguments have been fully considered but are not deemed persuasive. For the record, it is noted that there is no specific teaching or suggestion in any of the references cited indicating that the repair substrate and the nucleic acid encoding the chimeric nuclease should be separated onto separate vectors. There is not a single statement or teaching in any of the references cited indicating the unpredictability of using a single vector as claimed, nor there is any teaching or suggestion in any of the cited references regarding expression of a nuclease to interfere with access to the repair substrate or vice versa. With regard to the teachings of Choulika et al., it is noted that Choulika et al. teach a single vector comprising both the substrate and the nucleic acid encoding the nuclease. The relevant teachings of Choulika et al. regarding this issue have been reproduced below:

> [0044] Targeting DNA and/or restriction endonucleases introduced into a cell or individual as described above can be inserted in a vector. As used herein, a "vector" includes a nucleic acid vector, e.g., a DNA vector, such as a plasmid, a RNA vector, virus or other suitable replicon (e.g., viral vector).

> [0049] A vector comprising targeting DNA and/or nucleic acid encoding a restriction endonuclease can also be introduced into a cell by targeting the vector to cell membrane phospholipids. For example, targeting of a vector of the present invention can be accomplished by linking the vector molecule to a VSV-G protein, a viral protein with affinity for all cell membrane phospholipids. Such a construct can be produced using methods well known to those practiced in the art.

As indicated above, "targeting DNA" as used by Choulika et al. is equivalent to "repair substrate" as recited in the claims in view of the fact that Choulika et al. defines "targeting DNA" as comprising all the elements recited in the claims with regard to a "repair substrate". See discussion above under "Grounds of Rejection". From the teachings reproduced above, it is clear that Choulika et al. teach that

the targeting DNA and the nucleic acid encoding the restriction endonuclease can be placed in a vector (one single vector). If the argument is made that the term "a vector" should not be read as "one single vector", the reader's attention is drawn to paragraph [0049], where Choulika et al. state "a vector comprising targeting DNA and/or nucleic acid encoding a restriction endonuclease can also be introduced into a cell by targeting the vector to a cell membrane phospholipids" (emphasis added). Clearly, "the vector" as stated by Choulika et al. refers to a single vector. Therefore, it is abundantly clear that Choulika et al. teach a single vector comprising both the targeting DNA (i.e., repair substrate) and the nucleic acid encoding the nuclease.

While it is agreed that a vector comprising two nucleic acids encoding two chimeric nucleases and the repair substrate (claim 102) is not taught by the cited prior art, it is reiterated herein that the use of a single vector has one logical advantage which is found in the fact that having a single vector instead of two would reduce the number of transformations/transductions needed to introduce the desired nucleic acids in a host cell. Therefore, it is unclear to the Examiner as to how one of ordinary skill in the art would not be able to recognize that less experimentation is better than more. Having a single vector to deliver to a cell would require less experimentation than having to deliver two or more vectors to a cell.

Arguments stating that (1) the evidence establishes that the skilled artisan would have thought including both sequences on the same vector would complicate matters and be detrimental to their functions, (2) there is absolutely no motivation or expectation of success from the references that placing both sequences in one vector would be simpler or beneficial, and (3) there was no expectation that such vector would be functional, are found non-persuasive. Setting aside the fact that Choulika et al. teach a single vector comprising the repair substrate and the nucleic acid encoding the nuclease, as known in the art, and evidenced by Choulika et al. and Bibikova et al., vectors comprise both coding sequences and non-coding sequences (e.g., a promoter). For example, Choulika et al. teach that a vector comprises the desired coding sequence linked to one or more expression control sequences (non-coding) and that such

expression control sequences include promoter sequences, enhancers, and transcription binding sites (paragraph [0046]). It is well known in the art that plasmids, which are vectors, can comprise origins of replication (non-coding), antibiotic resistance coding sequences, sequences encoding purification tags (coding), sequences encoding signal peptides (coding), promoters (non-coding), termination sequences (non-coding), etc. Clearly vectors comprise more than one coding sequence and more than one non-coding sequence. Vectors which encode more than one protein are well known and widely used in the art. Therefore, it is unclear to the examiner as to how one of skill in the art can reasonably conclude that placing a coding and a non-coding sequence in a single vector would be detrimental to their function if vectors already carry several coding and non-coding sequences already.

If the "function" referred to by Appellant is related to its replication in the host cell, or its ability to be introduced in a host cell, there is no reason to believe that a vector comprising two nucleic acids encoding two nucleases (coding sequences) and a non-coding sequence (that of the repair substrate) would not replicate or could not be introduced in a host cell because, as stated above, vectors which are transferred to a host cell and replicate in a host cell that are able to express more than one protein are well known in the art (e.g., viruses). If the "function" is related to the expression of the nucleic acids encoding the nucleases, there is no reason to believe that those nucleic acids would not be expressed if the proper expression control elements are used, nor there is any reason to believe that the non-coding DNA (repair substrate) would inhibit the expression of the nucleic acids encoding the nucleases, unless the non-coding DNA is itself an expression inhibitor of the nucleic acids.

While Appellant has argued that the use of a single vector would complicate maters and be detrimental to their individual functions, Appellant has not provided any specific explanation as to how this could occur in the claimed invention, nor has Appellant provided any clue as to which functions would be negatively impacted by the use of a single vector. Contrary to Appellant's assertions, there is no evidence of record which teaches or suggests that using a single vector is in any way detrimental or

unpredictable. There is absolutely no indication in the specification that it would be unpredictable to use a single vector in their invention, or that the use of a single vector is a problem in the art which Appellant sought to solve. Paragraphs [0122] and [0138] of the specification state:

> "As described above, repair substrates and nucleases to be introduced into a cell can be inserted in a vector, and **optionally** a repair substrate and chimeric nucleases may be encoded on a **single plasmid**." (emphasis added)

> "Such methods may comprise the following steps: (a) introducing a chimeric nuclease into the cell, wherein said chimeric nuclease comprises: (i) a DNA binding domain; and (ii) a cleavage domain; and (b) introducing a repair substrate into the cell, wherein said repair substrate comprises: (i) a polynucleotide sequence that is substantially identical to a region on one or both sides of the target sequence; and (ii) a polynucleotide sequence which changes the target sequence upon recombination between the repair substrate and the target sequence. Upon recombination between the repair substrate and the target sequence, the target sequence is changed so as to match the repair substrate. **Optionally**, in such methods for gene targeting, the chimeric nuclease and the repair substrate are introduced into a cell on a **single vector**." (emphasis added).

Thus, one of skill in the art would reasonably conclude from appellant's own specification that the use of a single vector is another option to practice the invention but is not considered by Appellant to be a critical or unpredictable aspect of the invention. Therefore, in addition to the knowledge of one of skill in the art regarding vectors and introduction of vectors in a host cell, Appellant's own specification provide further evidence that there is no unpredictability in producing a single vector comprising two

coding sequences and a non-coding sequence, or in producing an isolated mammalian host cell comprising said single vector. While there is no absolute assurance that the claimed vector can be made or that one could introduced the claimed vector in an isolated host cell, there is a reasonable expectation of success at making a vector comprising two nucleic acids encoding nucleases and a DNA repair substrate, as well as at introducing such vector into an isolated mammalian host cell in view of the vast knowledge of the prior art regarding molecular biology techniques as well as the teachings of Choulika et al. and Bibikova et al. extensively discussed.

**II. Mammalian cells comprising repair substrates that are homologous endogenous chromosomal DNA are non-obvious over the cited references.**

On pages 7-10 of the brief, Appellant argues that the issue is not whether engineered zinc finger proteins were known to bind to endogenous genes but whether it was predictable at the time of filing from the teachings of Choulika and/or Bibikova that chimeric endonucleases comprising engineered zinc finger proteins and a cleavage domain would cleave endogenous DNA such that homologous recombination with the endogenous DNA was stimulated. According to Appellant, the evidence of record establishes that cleavage of non-endogenous chromosomal targets is not at all predictive of cleavage of endogenous chromosomal targets as claimed. Appellant refers to paragraph [0160] of the specification where it is stated that a GFP gene targeting system used by Appellant to study gene targeting (paragraph [0158]) cannot be used for endogenous genes because that system depended on the prior introduction of a Sce site into the target gene, thus a method to create sequence specific double-strand breaks (DSBs) in endogenous genes needs to be developed. Appellant submits that the specification teaches that a foreign sequence inserted into a chromosome was not considered an endogenous target and that it was not considered predictable at the time of filing that zinc finger nucleases would cleave endogenous chromosomal targets. Appellant asserts that Choulika et al. teach that the SceI binding site is not

endogenous to a mammalian genome and must be inserted for cleavage to occur. Appellant argues that since the reference by Choulika et al. predates all the cited art regarding zing finger protein engineering, it is clear that this reference does not establish predictability of using chimeric zinc finger nucleases to cleave endogenous chromosomal DNA. Appellant further criticizes Bibikova et al. and states that Bibikova et al. chose not to cleave endogenous chromosomal DNA because they were unsure if zinc finger nucleases would result in targeting of endogenous chromosomal DNA by a repair substrate. Thus, appellant contends that Bibikova et al. teach that it was not considered predictable to induce homologous recombination at an endogenous chromosomal target sequence cleaved with a chimeric nuclease.

Appellant's arguments have been fully considered but not deemed persuasive. As previously indicated, Choulika et al. teach a repair substrate that comprises a nucleic acid sequence which is homologous to the region surrounding the site to be targeted in chromosomal DNA. Choulika et al. also state that their invention relates to a method of repairing a specific sequence of interest in chromosomal DNA of a cell. Moreover, Choulika et al. teach that their invention relates to a method of correcting a genetic lesion in chromosomal DNA of a cell comprising inducing in the cell double stranded cleavage at a site of interest in the genetic lesion (paragraph [0011]). Thus, it is abundantly clear from the teachings of Choulika et al. that the intended target of Choulika et al. is endogenous chromosomal DNA. While it is agreed that the working example provided by Choulika et al. discloses that they inserted an I-SceI site, which is not normally found in the chromosome of the mammalian cell line they used (NIH 3T3, mouse), in the chromosome of their cell line to test their hypothesis, it is noted that (1) the teachings of Choulika et al. are not limited to the working example provided (see "Grounds of Rejection" above for a detailed description of the relevant teachings of Choulika et al.), and (2) the working example of Choulika et al. clearly demonstrates the principle that one could target the chromosome of a host cell for repair, thus at a minimum suggesting the "endogenous" limitation.

The Examiner disagrees with Appellant's assertion that the evidence of record establishes that cleavage of non-endogenous chromosomal targets is not at all predictive of cleavage of endogenous chromosomal targets as claimed. With regard to the teachings of paragraph [0160] of the specification cited by Appellant as evidence of unpredictability of cleavage of endogenous chromosomal targets, it is noted that those teachings merely state that the GFP system cannot be used because the SceI site is not present in endogenous genes. This is consistent with the knowledge of the art because endogenous genes are not expected to have that site. However, this in no way is evidence that endogenous chromosomal targets cannot be cleaved with a chimeric nuclease that can recognize a site which is present in an endogenous gene, particularly in view of the teachings of Bibikova et al. who clearly teach that zinc fingers can be designed to recognize arbitrary recognition sites as well as the prior art cited in Bibikova et al. as evidence to show that zinc finger combinations with novel specificities have been successfully developed prior to the publication date of the reference by Bibikova et al. (page 289, right column, last 8 lines).

The Examiner agrees that Bibikova et al. do not provide a working example of cleavage of an endogenous chromosomal target. However, Bibikova et al. laid out the experimental procedure to follow for chromosomal gene targeting. See page 296, left column, Applications to gene targeting. Thus, at a minimum, Bibikova et al. suggests cleavage of an endogenous chromosomal target. Furthermore, while it is true that Bibikova et al. disclose the need to test cleavage of genuine chromosomal targets, it is noted that prior to the earliest priority date of the invention (7/3/2003; see page 3, item 3 of the Office action mailed 5/13/2008), targeting of endogenous chromosomal DNA was known in the art, as admitted by Appellant in the specification. As stated in paragraph [0160] of the specification:

> "In the GFP gene targeting system the introduction of a DSB stimulated GT by >2000-fold and the absolute rate of gene targeting reached 3-5% when conditions were optimized. Such

> a system, however, depended on the prior introduction of a Sce site into the target gene and therefore can not be used for endogenous genes. To stimulate gene targeting at endogenous genes, a method to create sequence specific DSBs in those genes needs to be developed. Chimeric nucleases have such potential (Chandrasegaran et al., 1999, Biol Chem, 380:841-8). **Chimeric nucleases--fusions between zinc finger binding DNA binding domains and the endonuclease domain of the FokI restriction enzyme ("Fn")--can site-specifically cleave** naked DNA *in vitro* (Chandrasegaran et al., 1999, Biol Chem, 380:841-8), extra-chromosomal DNA in *Xenopus* oocytes (Bibikova et al., 2001, Mol Cell Biol, 21:289-97) and **chromosomal DNA in *Drosophila* (Bibikova, et al., 2002, Genetics, 161:1169-75). Applicants decided to try to extend this methodology to stimulate gene targeting in human somatic cells** (FIG. 3)." (emphasis added).

The reference by Bibikova, et al. (Genetics, 161:1169-75, 2002) was also cited in the IDS filed 2/17/2004 as reference CB. Thus, it is clear from the record as admitted by Appellant in the specification that cleavage of chromosomal DNA by a chimeric nuclease having a zinc finger DNA binding domain and the FokI cleavage domain was know in the art prior to the earliest priority date of the instant application.

On page 10 of the brief, Appellant refers to the reference by Porteus et al. (Nature Biotechnology 23:967-973, 2005) which was published after the references by Choulika et al. and Bibikova et al., and also after the earliest priority date of the instant application. Appellant refers specifically to a statement in Box 3, page 969 of that reference where it is stated that although several hundred different homing endonucleases with different recognition sites have been identified, the major limitation to using them in gene targeting is that most mammalian genes do not have recognition sites for them. Appellant also refers to a paragraph in the reference by Porteus et al. bridging pages 970-971 where the teachings of Urnov et al. (Nature 435:646-651, 2005) are discussed. According to appellant, Porteus et al. confirms that it was

not until 2005 (over two years after the priority date of the instant application) that zinc nucleases were actually shown to cleave endogenous targets. Appellant refers to a statement by Porteus et al. where it is indicated that future work will be needed to translate *in vitro* findings to *in vivo* applications and to determine whether zinc finger nucleases create undesired genomic instability. Appellant continues to reiterate that the evidence of record, including the references cited, shows that cleavage of endogenous chromosomal sites with zinc finger nucleases was not predictable.

With regard to the teachings of Porteus et al. regarding homing endonucleases and their use in targeting mammalian genes, it is noted that the claims are directed to chimeric nucleases that comprise zinc finger DNA binding domains. Therefore, the chimeric nucleases recited in the claims do not require DNA binding domains from homing endonucleases. Thus, the teachings of Porteus et al. regarding homing endonucleases are deemed irrelevant to the instant discussion.

The Examiner disagrees with Appellant's contention that it was not until 2005 that the first report of cleavage of endogenous chromosomal DNA targets by chimeric nucleases appeared. Urnov et al., as taught by Porteus et al., reported that designed ZFNs can cleave an endogenous human gene in cultured cells. See page 970, right column, first sentence of the section titled "ZFN-mediated gene targeting of the SCID gene". However, as taught by Porteus et al. on page 969, left column, first sentence of the section titled "ZFNs in model organisms", Bibikova et al. (Genetics, 161:1169-75, 2002) were the first ones to successfully target a genomic locus with designed ZFNs (abbreviation for zinc finger nucleases) and they targeted the yellow gene of *D. melanogaster* (reference 35 in Porteus et al.). Thus, Porteus et al. further corroborate Appellant's assertion regarding the knowledge of the prior art regarding the cleavage of chromosomal DNA with chimeric nucleases comprising zinc finger DNA binding domains and the FokI cleavage domain. With regard to arguments that Porteus et al. indicated that future work will be needed to translate *in vitro* findings to *in vivo* applications and to determine whether zinc finger nucleases create undesired genomic instability, it is noted that while it is agreed that the art is unpredictable with regard to

targeting endogenous genes *in vivo* (i.e., gene targeting in human beings), the instant claims are directed to an isolated mammalian cell (i.e., cultured cell) and a vector. As such, while *in vivo* considerations as discussed in the reference of Porteus et al. would be very relevant if the claims were directed to a multicellular organism, in the instant case, such considerations are not an issue because the claims are directed to an isolated mammalian cell. Therefore, contrary to appellant's assertions, neither the knowledge of the prior art nor the evidence of record establishes that cleavage of endogenous chromosomal targets in isolated host cells (i.e., cultured cells) by chimeric nucleases is unpredictable.

**(11) Related Proceeding(s) Appendix**

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

/Delia M. Ramirez/

Primary Examiner, Art Unit 1652

Conferees:

/Manjunath N. Rao /

Supervisory Patent Examiner, Art Unit 1647

/Andrew Wang/

Supervisory Patent Examiner, Art Unit 1656